CYST FORMATION IN THE GLOMERULAR TUFTS OF CERTAIN FISH KIDNEYS

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In an earlier study (Grafflin, 1933), the paradox, in an old specimen of daddy sculpin (*Myoxocephalus scorpius*), of a kidney showing many glomeruli anatomically but no glomerular function physiologically was satisfactorily explained. The glomeruli had been rendered incompetent by degenerative changes affecting both the vascular tufts and the neck segments, so that it was practically impossible to find a single glomerulus which on anatomical grounds could be considered functional. In young fish of the same species there was found adequate anatomical basis for the varying, but low, glomerular function which could be demonstrated physiologically. However, considerable degeneration was already present in the youngest specimens examined, and these changes became steadily more prominent with increasing age (as judged by weight). For a complete discussion of the glomerular changes noted, the original paper should be consulted. Some glomeruli, relatively quite infrequent, exhibited cystic cavities in their vascular tufts, and this is the particular problem which concerns us here.

> "Probably the most interesting glomeruli in this material are those showing what may be called a central cystic degeneration. In some instances the tuft shows a more or less spherical and very well-delimited cavity which is either entirely free from coagulum or shows it in only small amounts. These clear spaces show a wide variation in size. . . . In other tufts the cyst shows rather poor delimitation and considerable amounts of coagulum." (Grafflin, 1933, p. 65.)

At that time such cyst formation had not been observed in the glomeruli of any other species (fish or higher vertebrates). Although the specimens of *M. scorpius* showed rather numerous parasites in the kidney, all of the evidence indicated quite clearly that the parasitism played no rôle whatsoever in the observed glomerular changes. Under the circumstances, one would reasonably be led to the conclusion that the cysts represented one manifestation of the generalized process of glomerular degeneration. However, the following statement was made: "The

[1] Fellow of the John Simon Guggenheim Memorial Foundation (1934). The specimens of *Ophichthys, Crenilabrus* and *Corvina* were collected at the Stazione Zoologica, Naples, Italy. I wish to thank Professor R. Dohrn for his many kindnesses while I was a guest in his laboratory.

sharp delimitation of these central cysts in some cases suggests that we may even be dealing here with a malformation of the tuft" (p. 66). In the course of the last several years instances of cyst formation have been found in the glomerular tufts of seven additional species, two of them lungfishes, four of them marine teleosts, and one of them an arid-living reptile (the horned toad). None of these species exhibits the generalized glomerular degeneration characteristic of *M. scorpius*. Further discussion of the problem will be deferred until after a description of the findings.

Lungfishes

Protopterus æthiopicus (*African lungfish*)

In one of the available specimens (No. 21) two striking instances of cyst formation were observed, of widely different sizes and both exhibiting a delicate coagulum (Figs. 1 and 2). In another specimen (No. 34) two cysts were likewise observed (Fig. 3). Though closely adjacent, they are in different glomerular tufts, which are located, however, in the same glomerular cluster. In these latter instances the cyst contains much heavier coagulum, including what appears to be cellular debris, and in addition some well-formed cellular elements, presumably inwandering leucocytes. All four of these cysts are sharply delimited, and are lined by a flattened, endothelium-like epithelium (particularly well shown in Fig. 3). Immediately outside of this epithelial lining there is a well-defined basement membrane, which stands out quite clearly in Fig. 3. In comparison with non-cystic glomeruli, there is no increase in cellularity of the tuft, and no detectable abnormality of

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Explanation for Plate 1

Figs. 1 and 2. A large and a small cyst in glomerular tufts of *Protopterus æthiopicus*. Iron hematoxylin and orange G.

Fig. 3. Two cysts, side by side, in adjacent glomerular tufts of *Protopterus æthiopicus*. Heidenhain-azan. × 410.

Fig. 4. Parasite in glomerular tuft of *Protopterus æthiopicus*. Iron hematoxylin and orange G.

Fig. 5. Small cyst in glomerular tuft of *Lepidosiren paradoxa*. Iron hematoxylin and orange G.

Figs. 6, 7 and 8. Cysts in glomerular tufts of *Myoxocephalus octodecimspinosus*. Heidenhain-azan.

Fig. 9. Glomerular tuft of *Myoxocephalus octodecimspinosus*, showing extensive region of degenerative change, interpreted as probable precursor of cyst formation. Heidenhain-azan.

All sections 5 μ. All microphotographs at × 350 except Fig. 3. All cysts, except the small one to the left of Fig. 3, photographed at point of greatest cross-sectional area.

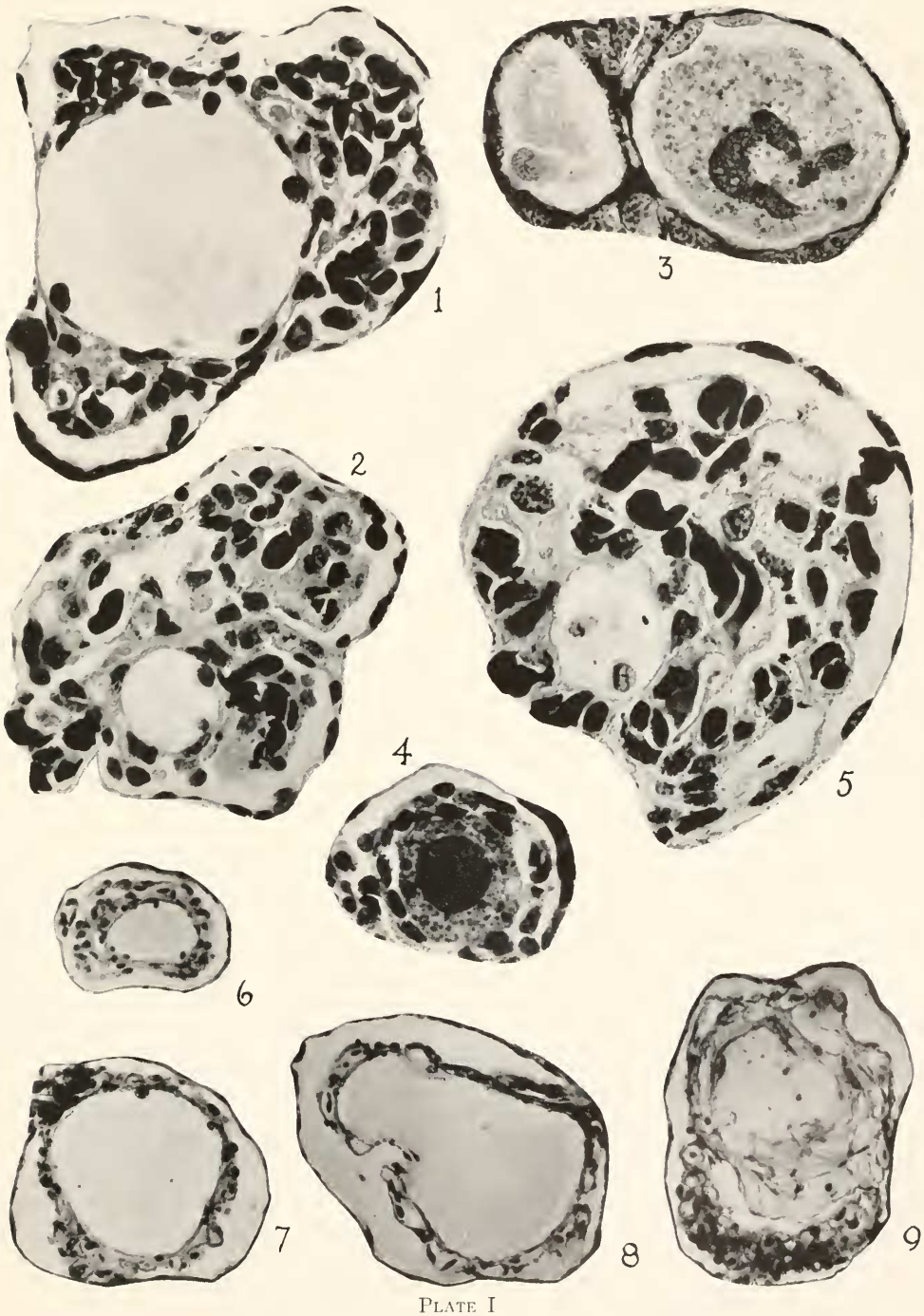

PLATE I

the afferent and efferent vessels or of the peripheral capillaries. The intracapsular spaces are free from coagulum, the ciliated neck segments are normal, and the first portions of the proximal convoluted segments are entirely comparable with those of adjacent nephrons showing no glomerular abnormality. In Specimen No. 21 the presence of a parasite in the glomerular tuft is a not infrequent finding. These parasites always exhibit a characteristic structure, which is shown in Fig. 4. Careful study of the material has failed to unearth any positive evidence that the parasites may ultimately be associated with the appearance of cysts such as those described above, but for the present such an association cannot be denied and must be left an open question.[2]

Lepidosiren paradoxa (South American lungfish)

In one of the specimens available a single small cyst was observed (Fig. 5). It is essentially similar in structure to the cyst shown in Fig. 2. It differs in having a less rigidly spherical outline and in containing, in addition to coagulum, a few formed cellular elements. The general statements made above concerning the cystic glomeruli of *Protopterus* likewise apply to this instance in *Lepidosiren*. Although parasitic remains are present in the kidney, they have never been observed in the glomeruli, and it is concluded that they have nothing to do with the formation of the cyst in question.

EXPLANATION FOR PLATE II

FIGS. 10, 11, 12, 13 and 14. Five instances of cyst formation in glomerular tufts of *Ophichthys imberbis*. 5 μ.

FIG. 15. Normal glomerular tuft of *Ophichthys imberbis*, without cyst formation, showing large intracapsular space frequently observed in the present material. 5 μ.

FIGS. 16 and 17. Isolated instances of cyst formation in glomerular tufts of *Crenilabrus pavo* (Fig. 16) and *Corvina nigra* (Fig. 17). 5 μ.

FIGS. 18 and 19. Two instances of cyst formation in glomerular tufts of the horned toad, *Phrynosoma cornutum*. 10 μ.

All sections stained with hematoxylin and eosin; all microphotographs at × 350; all cysts photographed at point of greatest cross-sectional area.

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[2] These specimens were collected in Africa by Professor Homer W. Smith. For the sake of completeness the following data are given. Specimen No. 21: collected in July, 1928; kept alive in dry estivation from November 1, 1928, for 427 days; replaced in water for ten days and killed, as it seemed about to die after the appearance of superficial infection. Specimen No. 34: collected in July, 1928; kept in water until January 10, 1930, being fed regularly; accidentally killed by exposure to cold. From a study of the available material, there is no evidence that a period of estivation has any bearing whatsoever upon the formation of cysts.

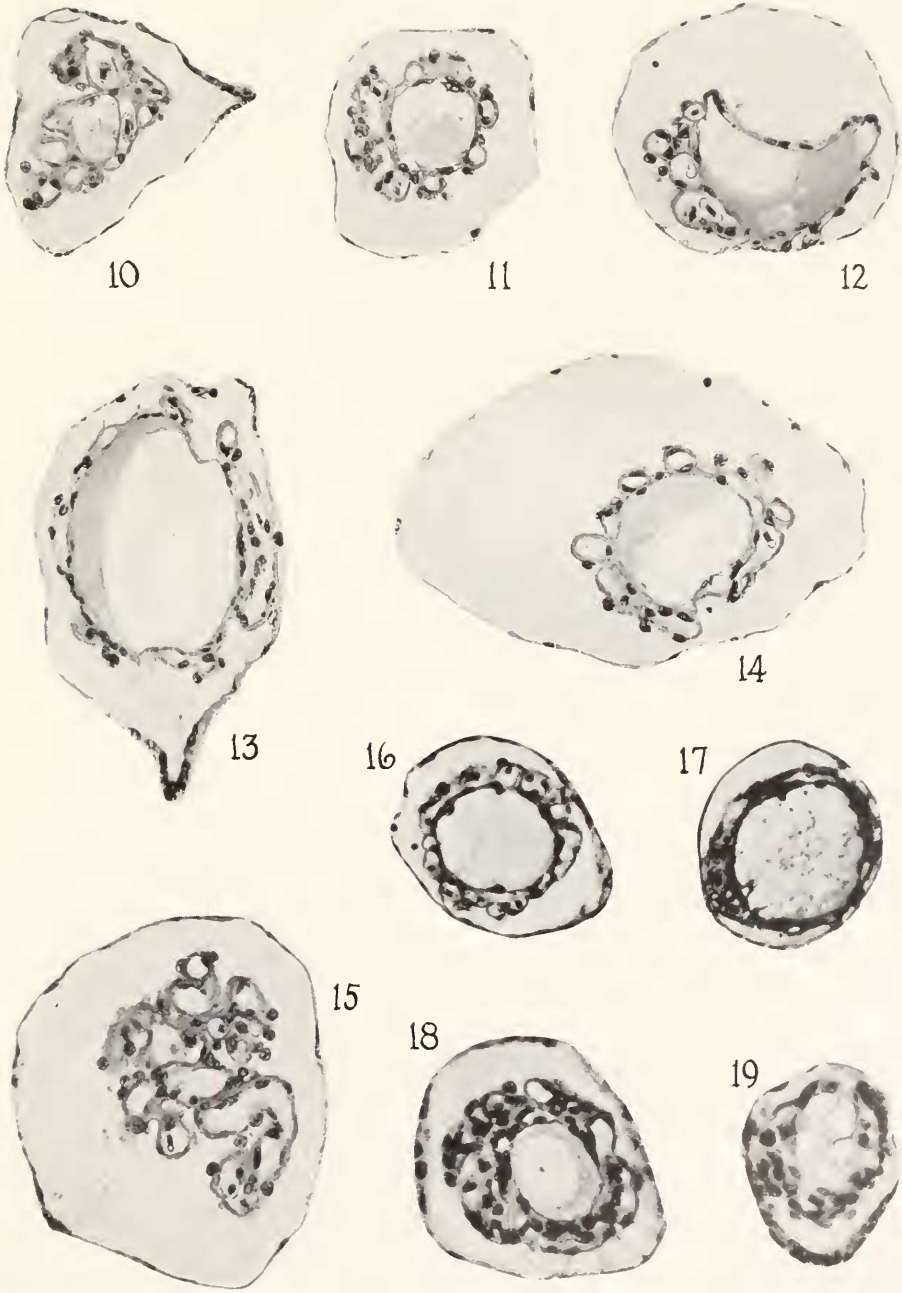

PLATE II

Marine Teleosts

Myoxocephalus octodecimspinosus (*sculpin*)

As reported in detail elsewhere (Grafflin, 1937), the glomeruli of this species exhibit a wide variation in size and vascularity, but are in no sense the seat of generalized degenerative processes such as characterize the glomeruli of the closely related daddy sculpin (*M. scorpius*). Nevertheless, glomerular cysts have been found to occur in all of the six specimens carefully examined. In five of these specimens such cysts are present in only relatively small numbers. In the sixth, in one restricted portion of the kidney, cystic glomeruli comprise almost one-third of the total glomeruli. No reason for their large number in this region is apparent. Three examples of cystic glomeruli, to show the range in size encountered, are given in Figs. 6, 7 and 8. The cysts are sharply delimited, are lined by a markedly flattened epithelium (the nuclei of which are particularly apparent in Fig. 6), and contain coagulum of varying density. As in the lungfishes, the intracapsular spaces are free from coagulum, and the afferent and efferent vessels and the peripheral capillaries are apparently normal, as are the associated neck and proximal convoluted segments. A careful search for some lead as to the mechanism by which these cysts might arise yielded only the isolated glomerulus shown in Fig. 9, which is nevertheless very striking. While the lower part of the tuft retains essentially normal glomerular organization, the bulk of the tuft has lost it completely, and exhibits for the most part a coarse, stringy meshwork containing numerous naked nuclei. It seems reasonable to suppose that the degenerative process, which is here so clearly apparent, might eventually result in the formation of a large cyst of the type shown, for example, in Fig. 8. Small unicellular parasites regularly occur in the sculpin kidney. However, since they are never found in the glomeruli, it is concluded that they play no rôle in the formation of the cysts.

Ophichthys imberbis

In material from the caudal kidney of the single specimen available for study (Naples—weight 28 grams), five instances of cyst formation have been observed (Figs. 10–14). In all cases the ciliated neck segment is fully patent and entirely normal in appearance, and the intracapsular space shows no trace of coagulum. That the relatively large size of the intracapsular space (see particularly Fig. 14) bears no essential relationship to the presence of the cysts is clear from an examination of the normal glomeruli of this specimen, in which this space is frequently unusually large (Fig. 15). The cysts vary in size, but are

all sharply delimited and are lined by a flattened epithelium. They contain a considerable amount of coagulum, which has withdrawn to one side of the cavity, presumably in the course of fixation. The capillaries persisting in the outer rim of the tuft are normal in appearance and fully patent, and the visceral epithelium is identical in thickness and appearance with that of the normal glomeruli. The afferent and efferent vessels are likewise normal. The cysts tend to be spherical in shape, but in one case (Fig. 12) the cyst wall has collapsed, perhaps in the course of fixation. The glomerulus shown in Fig. 12 is to be compared with one previously described in the daddy sculpin (Grafflin, 1933, Figs. 7 and 15).

In three instances (Figs. 11, 12 and 14) the ciliated neck can be readily traced into the first portion of the proximal convoluted segment, which is entirely normal in appearance. In another case (Fig. 10) the sections do not include the transition. In the fifth instance (Fig. 13) the ciliated neck, approximately 100 μ in length, passes into a curious segment with flattened cuboidal epithelium, which shows neither cilia nor brush border. The cytoplasm is scanty and lightly eosinophilic; the nuclei are closely packed but show no signs of degeneration. This segment persists for about 250 μ, at which point it shows a transition to the normal epithelium of the first portion of the proximal convoluted segment.

Crenilabrus pavo

In material from the caudal kidney of the single specimen available for study (Naples—weight 120 grams), a single instance of cyst formation was observed (Fig. 16). The cyst is spherical and sharply delimited, is lined by a flattened epithelium, and contains a moderate amount of coagulum. The peripheral capillaries and the afferent and efferent vessels are entirely normal in appearance, and the visceral epithelium is unthickened. There is a very delicate coagulum in the intracapsular space. The ciliated neck segment, which is very short, is fully patent, and the associated first portion of the proximal convoluted segment shows no abnormality.

Corvina nigra

The solitary instance of cyst formation observed in this species (single specimen, caudal kidney; Naples—575 grams) is illustrated in Fig. 17. The cyst is essentially spherical, is lined by a flattened epithelium, and exhibits a rather coarse, stringy coagulum. A few naked nuclei, fairly well preserved, are found scattered at random through the

coagulum. The peripheral capillaries, few in number, contain normal red cells, and the afferent and efferent vessels show no detectable abnormality. The glomerular membrane is in many places appreciably thickened. The neck of the tubule is patent, though the lumen is quite small, and the first portion of the proximal convoluted segment is entirely normal in appearance.[3]

Horned Toad (Phrynosoma cornutum)

In surveying the available sections of the kidney of the horned toad, which is an arid-living reptile, two instances of cyst formation, entirely comparable with those observed in the fishes, were found (Figs. 18 and 19). Only a portion of the glomerulus shown in Fig. 18 is present in the sections, and both the afferent and efferent vessels and the neck segment are absent. The cyst is spherical and well-delimited, is lined by a flattened epithelium, and contains a considerable amount of coagulum. In the two sections adjacent to the one photographed there is present a dense, irregular, deeply basophilic mass of debris, which occupies perhaps one-quarter of the total cross-sectional area of the cyst. The peripheral capillary loops contain normal red cells, and show no thickening of the glomerular membrane as compared with the normal. There is a distinct coagulum in the intracapsular space. This glomerulus has formerly been briefly noted by Vilter (1935, p. 383). The appearance of the cyst in Fig. 19 is deceptive, due to the manner in which the coagulum has been precipitated. Actually the cyst is sharply delimited from the surrounding tissue, and is lined by flattened epithelium. The glomerular membrane is not thickened, and the peripheral capillary loops contain normal blood cells. The afferent and efferent vessels seem entirely normal, and the neck segment is fully patent.

Discussion

In seeking for an explanation for the formation of the cysts described above, we are led to the following considerations:

(1) All of the evidence indicates quite clearly that the presence of parasites in the kidney has no bearing upon the formation of cysts, except in the case of one specimen of *Protopterus* (No. 21). In this specimen, the tendency of the parasites to locate in the glomerular tuft is suggestive. The parasite might become walled off, and, with the sub-

[3] In this specimen one small, degenerate avascular tuft was observed which showed a central cavity containing basophilic debris. The peripheral rim of tissue was hyalinized and almost structureless, still containing scattered nuclei and nuclear fragments. This cavity is in no sense typical of the cysts discussed here, and will not be further considered.

sequent evacuation, or degeneration and absorption, of the organism, cystic cavities of the type observed might persist. However, there is no direct evidence in the material at hand that such is the case. From the available evidence, it is concluded that the glomerular cysts are formed predominantly, or entirely, on some basis other than parasitism of the glomerular tuft. If parasitism can play a causative rôle, it is a completely separate process and of minor importance for the present problem.

(2) It seems almost certain that at least some of the cysts are formed as the result of a degenerative process in the glomerular tuft. In favor of this view are some of the pictures observed in the kidney of *Myoxocephalus scorpius* (Grafflin, 1933) and the striking glomerulus observed in *Myoxocephalus octodecimspinosus* (Fig. 9).

The two instances of cyst formation in glomerular tufts of the horned toad are particularly interesting. In the first place, this is the only species above the fishes in which such cysts have yet been recorded. In the second place, the reptilian glomerulus usually exhibits a central, avascular, cellular core, which, according to Regaud and Policard (1903) and Cordier (1928), is made up of connective tissue. Such a core is constantly present in the glomeruli of the horned toad (Marshall and Smith, 1930; Vilter, 1935). In the two glomeruli illustrated in Figs. 18 and 19, the cystic cavities occupy the region of the typical central cellular core, and replace it to such an extent that no characteristic portion of the core is any longer recognizable. One is led to wonder whether the cysts might not have arisen as the result of degeneration of the central avascular area. The irregular mass described above for the larger cyst (Fig. 18) is opaque and amorphous, and has all the appearance of calcified debris; it might be construed as the remains of the original core.

Let us now examine the available fish material in the light of these considerations for the horned toad. The glomerular tufts of *Crenilabrus* tend to be somewhat cellular, and one occasionally finds a central avascular core. The tufts of *Corvina* tend to be quite cellular, and it is not infrequent to find a typical central cellular core, entirely comparable with that seen in the horned toad and pigeon (see below). In *Myoxocephalus scorpius* (Grafflin, 1933) many tufts show a markedly cellular center, which may be entirely avascular. In an earlier study (Grafflin, 1929) it was shown that the relatively few glomerular structures present in the kidney of the adult goosefish (*Lophius piscatorius*) have lost all connection with renal tubules. The important fact for the present problem is that eight out of thirty-one of these

"pseudoglomeruli" which were carefully studied showed a central degeneration of the glomerular tuft. In seven of them the center of the tuft was hyaline, eosinophilic and entirely avascular, and showed a few scattered nuclei and nuclear fragments; in two of these seven this central area was vacuolated in addition.[4] In all of these tufts the central hyaline area was very sharply delimited from the peripheral tissue.

If our well-delimited cysts are to be interpreted in terms of degeneration and liquefaction of a central avascular portion of the tuft, certainly it is just as conceivable that such a process could occur in all of the fishes described above as in the horned toad. In favor of this interpretation are the amorphous mass in one of the cysts of the horned toad and the scattered nuclei in the cyst of *Corvina*.

However, ranged against such an interpretation are the following facts. (1) The two cysts observed in the horned toad are isolated instances, whereas one might reasonably expect them to be numerous on this basis. (2) In the pigeon, whose glomeruli likewise exhibit a cellular avascular core, an extensive search of abundant material failed to reveal a single instance of cyst formation (Vilter, 1935). (3) Though many glomeruli of the goosefish show marked degeneration of the central part of the tuft, no instance of cyst formation in such a tuft has yet been observed. (4) In the specimens of *Ophichthys, Lepidosiren* and *Protopterus,* the glomerular tufts are well vascularized, and no accumulations of cells have been observed which in any way suggest a cellular avascular core. Similarly in the sculpin (*M. octodecimspinosus*), in the usual range of glomerular size, no well-defined central core has yet been observed. (5) Some of the cysts are very small, and it seems perfectly clear that as we see them they are at their maximum size. It is hardly conceivable, in view of the findings in the pigeon, that an avascular region of the size represented by these small cysts would undergo degeneration. (6) The cysts are in general spherical, and give every indication of having contained fluid under pressure. If we were dealing merely with a degeneration of the central portion of the tuft, one would more logically expect collapse of the tuft rather than distension of the type observed.

In summary, while some of the glomerular cysts are apparently formed on the basis of a degenerative process in the glomerular tuft,

[4] The eighth tuft was very small and atrophic, and showed a central degeneration to the point of cavity formation, the cavity containing granular debris. The picture is not at all typical of the cysts discussed here, and will not be further considered.

In the legend to Fig. 7 of this earlier paper there is an obvious error. It is clear from the illustration that no ciliated neck segment is present, and that the intracapsular space opens directly into a segment whose cells exhibit the brush border characteristic of the epithelium of the proximal convoluted segment.

it seems unlikely that all of the cysts which we have observed could arise in this manner.

(3) It is suggested that many of the glomerular cysts herein described probably result from a malformation of the glomerular tuft. The cyst might be laid down early in embryological development, or it might be formed rather late, when glomerular organization is already well advanced. In the latter case two possibilities immediately suggest themselves: (*a*) the walling-off of a deep crevice between adjacent lobulations of the tuft; (*b*) the occlusion at both ends of a portion of one of the glomerular capillaries. On either basis one could readily understand (1) the flattened epithelial lining of the cyst; (2) the subsequent enlargement of the cyst without, at the same time, any encroachment upon or collapse of the surrounding capillaries; and (3) the presence within the cyst of coagulum, which would represent simply a seepage of plasma proteins into the completely closed cavity. Also, such a mode of formation would be consistent with the small size of some of the cysts and the failure to find, in adult animals, more than occasional suggestive intermediate stages in cyst formation.

Summary

Well-delimited glomerular cysts have been observed in the kidneys of the following species of fishes: *Protopterus æthiopicus, Lepidosiren paradoxa, Myoxocephalus scorpius, Myoxocephalus octodecimspinosus, Ophichthys imberbis, Crenilabrus pavo, Corvina nigra;* and in the horned toad, *Phrynosoma cornutum.* It is concluded that these cysts are probably formed in two ways: (1) on the basis of a degenerative process in the glomerular tuft; (2) as the result of a malformation of the glomerulus in embryological development.

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